Commentary

Thinking About Cancer Causality and Chemoprevention[1,2]

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Chemoprevention is a strategy used to block the development of cancers in humans. Necessarily, the approach draws its rationale from a multitude of disciplines. The interdisciplinary milieu necessary to conduct chemoprevention research has muddied, if not obscured to nonaficionados, the scientific basis of chemoprevention and its relationship to more established scientific endeavors. Figure 1 presents chemoprevention in terms of the relationship of cancer etiology and biology to prevention and evaluation of diesase end points.

Chemoprevention interfaces with biology, epidemiology, and behavioral sciences, with clinical trials serving as the tactical arm of experimental implementation in humans (*1*). In this commentary, the implications of concepts and relationships associated with these interfaces are elucidated through a series of questions. The answers to these questions will impact on the scientific development of chemoprevention over the next decade.

Questions

What will be the contribution of genetic background to designing chemoprevention trials?

To date, cancer intervention trials have been based on risk characteristics of individuals in a population, and subsequent trials have measured the outcome for the population of individuals in the arms of the trial. Consequently, trials are designed to include large numbers of individuals with relatively long-term exposure to the intervention. This approach has been used to assure that trial data are adequate to demonstrate the significance and power necessary to prove the validity of study conclusions. There is increasing evidence that cancer risk is intimately associated with genetic structure at the molecular level (2). Advances in analysis of the molecular genotype using restriction fragment-length polymorphisms and other approaches have made identification of hereditary diseases at the molecular level possible; the same approach is likely to be used in the study of most cancers soon. Investigators have identified genetic polymorphisms that appear to give individuals substantially increased risk for particular cancers. Almost certainly, with continued advances in molecular biology, epidemiologic studies will further establish actual baseline risks for most diseases. Phenotypic expression of the disease will be shown to range from absolute (e.g., bilateral retinoblastoma) to unlikely, depending largely on susceptibility of the underlying molecular structure to permanent mutagenic damage by any particular initiator.

On the basis of these findings, individuals with a high genetic risk should be identifiable, and the required sample size for any particular trial should be markedly reduced. Individuals at high risk for a disease would be excellent candidates for a specific primary prevention trial. In addition, advances in detection of DNA damage (e.g., DNA adduct formation) may allow identification of individuals who have both high risk and mutagenic changes in the relevant tissue.

What is the role of epidemiology in a biological model of disease prevention?

Epidemiologic approaches have identified major dietary components and cultural practices that affect the phenotypic expression of many cancers. Additional study of these factors in individuals at risk, or perhaps in vitro at the cellular level, may allow new insights into the fundamental processes and influences operative at the phenotypic level. A better understanding of the interaction of risk factors and specific genes should result in a new mechanistic epidemiology at the molecular level. For example, certain genetic changes

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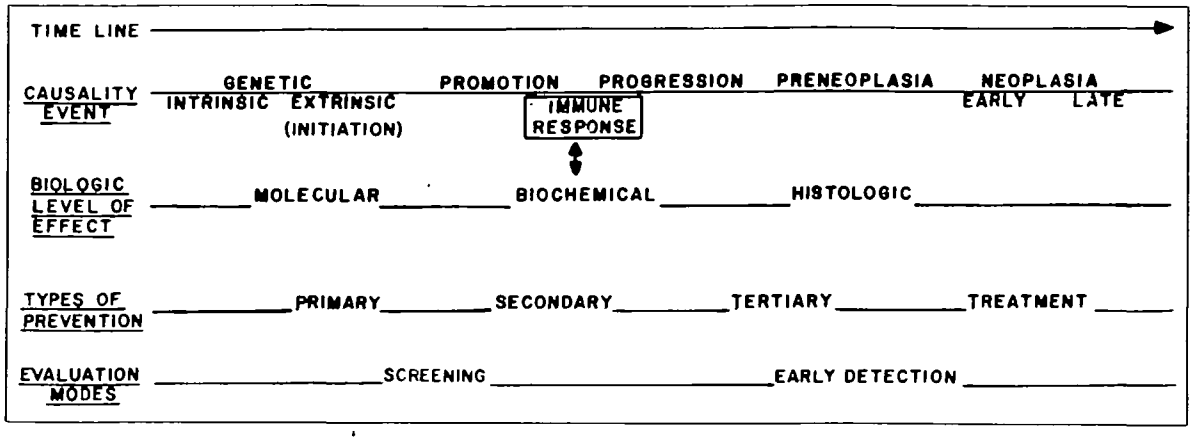

Figure 1. Relationship of cancer causality and biology to prevention and evaluation of disease end points.

may result in functioning proteins that exhibit subtle differences from the normal and interact differentially in particular phenotypes to produce an enhanced risk for abnormal proliferation (*3*).

Another unexplored area is the epidemiology of preneoplastic growth, which offers a rich opportunity for novel research. Little is known about what causes preneoplastic or metaplastic lesions to form or to evolve into frank malignancies. It is known, however, that cigarette smoking, exposure to ultraviolet light, and use of alcohol enhance the progression of promoted and preneoplastic cells to the malignant phenotype in appropriate tissues.

What new approaches should be explored for inhibiting carcinogenesis?

New approaches to the inhibition of carcinogenesis include the use of anti-initiators, antipromoters, and antiproliferative agents that show promise for chemoprevention in humans (*1,4*). Another approach is the interaction of early immune response with phenotypically altered cells, an area of study that has been almost totally neglected. The results of sophisticated experimental studies using ultraviolet irradiation of skin and analysis of host immune and nonimmune cell distribution in skin lesions indicate that complex interactions occur between immune cells and damaged normal cells (*5,6*). Undoubtedly, subtle changes in host immune status occur in response to the first biochemical membrane lesion or altered cytokine release, and crude pharmacologic approaches can be used to interfere with these processes. It is likely that with more detailed understanding of the biological battlefield, specific pharmacologic or biologic agents will be developed to interfere with the escape of early precancerous growth from host immune control without producing irreversible effects on the host immune system.

What is the natural history of premalignant conditions?

An understanding of the biology of preneoplastic lesions should provide important information about carcinogenesis in humans. The study of the progression of carcinogenesis is relatively new and is essentially confined to animal cutaneous models. Except for cervical dysplasia (*7*), very little is known about the biology of premalignant conditions in humans. Investigators have begun to study the precursive biology of cutaneous melanoma (*8*), esophageal cancer (*9*), and to a lesser extent, colon cancer (*10*), but virtually nothing is known about the biology of these lesions or of such lesions as oral leukoplakia, bronchial metaplasia, gastric metaplasia, and actinic keratosis.

Are there good intermediate markers for cancer or cancer risk?

The extensive time required for progression from the damaged cell to cancer in humans has made the study of intermediate biochemical markers attractive. However, a major problem is that we do not know if any of them are valid indicators of cancer risk or development. Most investigators have used markers to measure abnormalities by analysis of DNA content or cytogenetic study or by indirect [(ornithine decarboxylase or protein kinase C (PKC)] or direct (Ki67 antibody, thymidine labeling, or mitotic index) estimation of proliferation. Chromosomal abnormalities have been predictive of malignant outcome, but in general, they have been identified at late stages of the disease (*11,12*). Although several complex studies have used intermediate markers for colonic cell proliferation, including favorable modulation by calcium, it has not been demonstrated that these changes are indicators of cancer risk or development (*10,13,14*). The concept of intermediate markers is valuable for the study of human neoplasia and of the modulating effects of chemoprevention agents, but more detailed investigations in animal models and in human disease are needed to validate the approach.

What are desirable properties of a chemoprevention agent in humans?

The optimal chemoprevention agent would be nontoxic, highly efficacious, easily measurable in serum and tissue, and readily available. For the most part, dietary components such as β-carotene, α-tocopherol, and vitamin C fill this bill. Other dietary elements such as selenium appear to have a steep efficacy/toxicity ratio. With few exceptions, the pharmacological compounds proposed as chemopreventive agents have side effects that are dose related. The acceptability of side effects depends on the nature of the condition being studied. An individual with familial polyposis may be willing to accept considerable side effects from a chemoprevention agent. In contrast, a person 55 years of age who has had one polyp removed in the past year and is asked to join a

chemoprevention trial to prevent polyp recurrence may express considerable reservations if any side effects from a drug are expected. One way to reduce side effects is to determine the lowest dose at which an agent will produce a significant and relevant biochemical or biological effect. Careful phase I studies with modulation of biological or biochemical end points in relevant tissues are needed to provide the basis for planning future chemoprevention trials.

What is the biological rationale for development of chemoprevention agents?

There are a number of ways in which chemoprevention agents can be viewed. One possible unified approach is shown in figure 2. The common strategy for chemoprevention at the cellular level is modulation of the key regulatory pathways, most notably, signal transduction and control mediated by the phosphatidylinositol cascade, the cyclic nucleotide pathway, and polyamine regulation. The intricacies of these regulatory controls have been described in detail (*15-17*). The purpose of this discussion is to point out new possibilities for chemoprevention strategies in the future.

A multitude of growth factors mediate the effects of these regulatory controls by signal transduction via the phosphatidylinositol (fig. 2, A1) cascade or the cyclic nucleotide (fig. 2, A2) pathway. Undoubtedly, abnormalities in growth factor production, growth factor receptor function, or integration of the response will be found to be early changes in malignant transformation. Abundant data indicate that growth factors can either produce or inhibit proliferation in the same cells, depending on the biochemical and cellular milieu (*18*). The development of signal analogs or inhibitors that differentially affect receptor function should lead to the availability of new chemoprevention agents as well. Propagation of the signal via the phosphatidylinositol pathway requires a steady source of inositol (fig. 2B). The regeneration of inositol from inositol-1-phosphate is selectively inhibited by lithium and results in abrogation of signal responses (*19*). Lithium carbonate (Eskalith) has been widely used as a drug for manic-depressive illness; the pharmacology is well known, and side effects closely correlate to serum levels. Consequently, further exploration of lithium as a potential chemoprevention agent is warranted.

The role of PKC in modulating cellular responses has been extensively studied, and the enzyme has a multiplicity of secondary modifications via phosphorylation. With the recent identification of these numerous subtypes of PKC, the possibility of interactions and modifications of key enzymes and receptors is nearly limitless. Because 12-*O*-tetradecanoylphorbol-13-acetate (TPA) has been identified as the ligand for PKC, the design of specific inhibitors seems possible and likely. Recent studies with the nonphorbol tumor promoter bryostatin suggest that the control of the enzyme is complex and the opportunity to develop specific modulators extensive (*20*). A number of nonspecific regulators (anti-inflammatory steroids) and selective regulators [H-series derivative (isoquinolinesulfonamides) and palmitoyl carnitine] of PKC have been identified and studied. It may be useful to study the effects of carnitine (fig. 2C), since the action of this natural substrate for palmitoyl carnitine and lipid biosynthesis has been well studied (*21*). Additionally, carnitine is commercially available for the treatment of inherited carnitine deficiencies.

An important intermediate in the phosphatidylinositol cascade, arachidonic acid, is the source of prostanoids, which are major stimulators of cellular proliferation in the cells of many tissues. Many nonsteroidal anti-inflammatory compounds inhibit prostaglandin synthesis. A particularly useful compound is proxicam (fig. 2D), which has been active as a chemoprevention agent in animal systems (*22*). The clinical experience with this drug has been extensive, and the spectrum of side effects at different doses is well defined.

One of the sentinel regulatory enzymes that undergoes

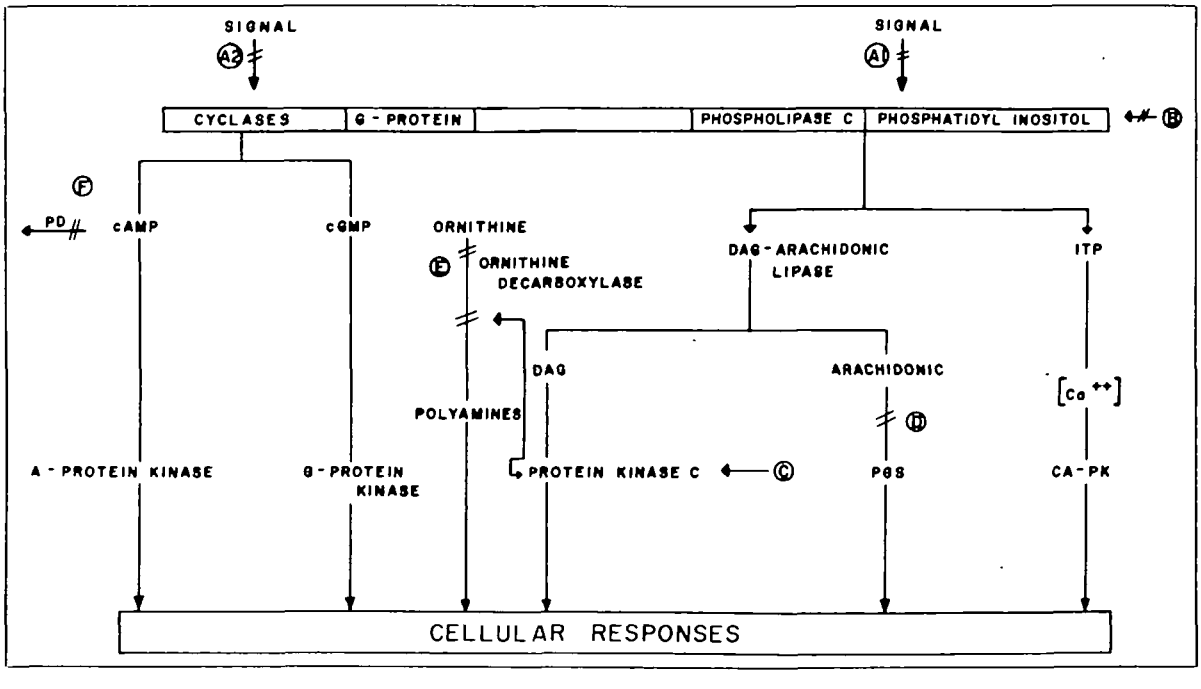

Figure 2. Diagram of a biological strategy for chemoprevention. Some potential inhibitors of carcinogenesis: A1 and A2, signal analogs; B, lithium; C, carnitine; D, piroxicam; E, DFMO; and F, theophylline. PD = phosphodiesterase; DAG = diacylglycerol; ITP = inosine triphosphate; PGS = prostaglandins; and CA-PK = calcium-activated protein kinase.

modification by PKC is ornithine decarboxylase, an important key enzyme in the polyamine synthesis pathway. The role of polyamine synthesis in cellular regulation has been extensively studied and recently reviewed (*16*). An important step in understanding the role of polyamines in cellular growth was the synthesis of an enzyme-activated irreversible inhibitor of ornithine decarboxylase activity, difluoromethylornithine (DFMO). This compound has been shown to be an inhibitor in all of the animal systems tested, whether it was applied topically or administered systemically. It has also been demonstrated that low doses of DFMO inhibit TPA-promoted human skin ornithine decarboxylase (*23*). Since this compound is almost nontoxic at low concentrations, its use as a chemoprevention agent in humans should be widely explored (fig. 2E). We have just started studies with DFMO (Eflornithine) in patients with oral leukoplakia and Barrett's esophagus and eagerly await the results.

The cyclic nucleotide pathway has been a rich target for study of differentiation and proliferation in normal and abnormal cells (*17*). In some systems, merely raising the cAMP levels by using theophylline (or derivatives) to inhibit the breakdown of cyclic nucleotide produces profound changes, with marked inhibition of growth and terminal differentiation. In other systems, no such effect is seen. Perturbation of this critical pathway as a strategy for chemoprevention has not been well studied at the experimental level. In view of the rigid controls evidenced by the study of this pathway, the development of modulators appears to be an important chemoprevention strategy (fig. 2F). The real possibilities for new and novel chemoprevention strategies based on an appreciation of key regulatory events in the cell should be evident.

In the past decade, the results of epidemiologic studies and clinical trials have provided the basis for a reliable scientific strategy for early intervention against human cancers. In the next decade, modern biology should also contribute information critical to the success of that endeavor. Epidemiology and biology as complementary underpinnings for chemoprevention in humans should become the order of the day.

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